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Effects of Ethanolic Leaves Extract of *Irvingia gabonensis* on Arsenic Trioxide-Induced Liver damage in Wistar rats

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ABSTRACT: The toxicity of drugs and other related agents attracts considerable attention from basic scientists to clinicians. Humankind has been passionate about the availability of cheap and readily available agent against organotoxicity. Therefore this paper investigated the effects of ethanolic leaves extract of *Irvingia gabonensis* on arsenic trioxide-induced liver damage in Wistar rats using appropriate standard methods. The results show administration of arsenic trioxide caused significant ($P<0.05$) serum increase in the activities of alanine aminotransferases and alkaline phosphatase (ALT and ALP) of the Wistar rats. There was significant ($p>0.05$) decrease in the activities of serum aspartate aminotransferases (AST) after the administration of arsenic trioxide. There were also significant ($p<0.05$) reduction in the serum level of total protein, albumin and globulin and statistically significant ($p>0.05$) increase in serum level of total bilirubin after administration of arsenic trioxide to the rats. This suggests damage to liver by arsenic trioxide, which are further supported by the histopathological findings such as periportal infiltrates of inflammatory cells, vascular ulceration, ductal epitheliosis and focal necrosis. Administration of extract of *Irvingia gabonensis* ameliorated both the histopathological and biochemical manifestation in the liver. In conclusion, the results from the histopathological and biochemical analyses suggested damage caused by arsenic trioxide and the potential of extract of *Irvingia gabonensis* in ameliorating the liver damage.

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Bush mango (*Irvingia gabonensis*) is a tree native to West and Central Africa. Its fruit, known as a dika nut in Hausa, Ogbono in Igbo and Oro in Yoruba, is commonly consumed in those regions and has gained popularity for its potential health benefits (Agbor, 1994). It is rich in fiber, vitamins, minerals, and antioxidants. The seeds of the fruit, commonly referred to as "seednuts," are high in fat and protein. Ethnomedicinal treatments utilize the bark, kernels, leaves, or roots for a variety of ailments. The bark is mixed with palm oil for treating diarrhea and for reducing the breast-feeding period. The shavings of the stem bark are consumed by mouth to treat hernias, yellow fever, and dysentery, and to reduce the effects of poison in French Equatorial Africa (Ejiofor and Okafor, 1997). The antibiotic properties of the bark help heal scabby skin, and the boiled bark relieves tooth pain. *Irvingia gabonensis* leaf and root extracts have documented inhibitory activity against several bacteria and fungi. Potential mechanisms of action include membrane disruption by terpenoids and inactivation of microbial adhesion, enzymes, and cell envelope transport proteins by ellagic acid-like compounds (Okolo *et al*., 1995**).** It contains various phytochemicals, including flavonoids and polyphenols, which are known to possess antioxidant

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activity (Okolo *et al.,* 2015). These compounds can scavenge and neutralize free radicals, thereby reducing the potential damage caused by oxidative stress. *Irvingia gabonensis* may also help stimulate the body's own antioxidant defenses. It has been reported to increase the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx). These enzymes play a crucial role in neutralizing and detoxifying free radicals (Jeema *et al.,* 2017). Arsenic trioxide (As_2O_3) is a highly toxic white crystalline compound whose ingestion or inhalation of arsenic trioxide has been reported to have harmful effects such as vomiting, diarrhea, shortness of breath, and headache (Aposhian, 2016). Severe side effects may include acute promyelocytic leukemia differentiation problems and cardiovascular problems such as: hypertension, artherosclerosis, endothelial dysfunction, oxidative stress, inflammation and arrhythmias (Lee and Harrison, 2009). Arsenic trioxide commercial applications are limited by its toxicity. It is a precursor to most pesticides, in glass manufacture and also used as a wood preservative (Engel *et al.,* 2004). It has been shown that ingestion of arsenic trioxide for long as a medical treatment (anticancer) can lead to skin cancer and reproductive problem. The effects of arsenic trioxide exposure can vary depending on the duration and concentration of exposure, as well as individual susceptibility factors. It's important to note that arsenic trioxide is a highly toxic compound, and precautions should be taken to minimize exposure. Chronic exposure to arsenic trioxide, especially through drinking water contaminated with arsenic, can lead to long-term health complications. This includes an increased risk of various cancers, such as lung, bladder, skin, liver, and kidney cancer. Chronic exposure may also contribute to the development of cardiovascular diseases, neurotoxicity, and reproductive disorders (Wang *et al.,* 2020). Furthermore, accidental ingestion of arsenic trioxide or consumption of contaminated food or water can lead to poisoning. Symptoms of arsenic trioxide ingestion may include abdominal pain, nausea, vomiting, diarrhea, and dehydration. The gastrointestinal effects can be severe and potentially life-threatening. Direct skin contact with arsenic trioxide can result in irritation and dermatitis (Wang *et al.,* 2020). Previous studies have demonstrated the occurrence of myocardial disorganization, interstitial oedema and infiltration of inflammatory cells in the heart and liver following exposure to arsenic trioxide in laboratory animals (Raghu *et al.,* 2009). It also resulted in a significant increase in the activity of certain relevant clinical enzymes for cardiac function and antioxidant mechanisms like the serum creatine kinase isoenzyme, lactate dehydrogenase, glutathione peroxidase and reduced glutathione (Raghu *et al.,* 2009). Arsenic trioxide has a long history and it is recognized as both poison and drug for more than two thousand years (Wang *et al.,* 2020). .The use of herbal therapy has received widespread attention within the global healthcare system because some pharmaceutical medications are based on a single active ingredient derived from a plant source. The importance of this study is to assess the effects of ethanolic leaves extract of *Irvingia gabonensis* on arsenic trioxide-induced liver damage in adult Wistar rats.

MATERIALS AND METHODS

*Collection, Identification and Preparation of Plant Material***:** Fresh *Irvingia gabonensis* leaves were harvested in Ekosodin community in Ovie North-East local government area of Edo State. It was identified and authenticated in the Department of Plant Biology and Biotechnology, University of Benin, Benin city. The leaves were thoroughly washed with tap water, air-dried and then pulverized to powdered form. The powdered form obtained was weighed and soaked in ethanol for 72 hours. The crude ethanolic extract was filtered using a Buchner funnel and Whatman No.1 filter paper. The filtrate was freeze-dried with freeze drying machine and refrigerated at -4 Celsius until use.

*Experimental Animals***:** Wistar rats weighing 160-190 g were procured from the Animal House, Department of Anatomy, University of Benin. Water and food were provided *ad libitum*. They were exposed to controlled environmental temperature (28 ± 2°C), relative humidity (50 ± 5%) and 12 hour light or dark.

Drugs Preparation: Arsenic trioxide (hepatotoxicant) that was used for this study was administered orally to Wistar rats at a dose of 10 mg/kg body weight. The plant extract was administered orally to Wistar rats at the doses of 250 and 500 mg/kg body weight.

Animal Groupings: Rats were randomly divided into six groups of seven animals each.

Group A: (Control) received only 1ml of distilled water daily for a period of 28 days.

Group B: (arsenic trioxide –induced) received 10 mg/kg body weight of arsenic trioxide orally for a period of 28 days.

Group C: received 250 mg/kg body weight ethanolic leaves extract of *Irvingia gabonensis* orally for a period of 28 days.

Group D: received 500 mg/kg body weight ethanolic leaves extract of *Irvingia gabonensis* orally for a period of 28 days.

Group E: received 250 mg/kg body weight ethanolic leaves extract of *Irvingia gabonensis* and 10 mg/kg

body weight of arsenic trioxide orally for a period of 28 days.

Group F: received 500 mg/kg body weight ethanolic leaves extract of *Irvingia gabonensis* and 10 mg/kg body weight of arsenic trioxide orally for a period of 28 days.

Administration was done using orogastic tube for 28 consecutive days. All the animals were then sacrificed the following day. During sacrifice, cotton wools were soaked with chloroform of about 30ml in an enclosed container and each rat was put in the enclosed container with chloroform for about 2-5sec for anaesthesing. After anaesthesing, the rat was placed on supine position on the dissection table (trolley). Abdomino-thoracic incision was made on the rat to expose the abdominal viscera. Thereafter blood samples were collected through inferior vena cava and through the heart by the process of venous and cardiac puncture respectively using 5mls syringes. The blood samples were turned into plain bottles and sera were obtained from it by allowing it to stand for 2 hours at room temperature, followed by centrifuging at 2000 rpm for biochemical parameters. Thereafter, the liver was harvested, dissected and immediately fixed in 10% forma-saline in a universal bottle for histological analysis.

*Histological analysis***:** The liver was examined grossly in all the dissected rats and was preserved in 10 % buffered formalin, dehydrated in ethanol (70 to 100 %), cleared in xylene and embedded in paraffin. All tissue sections were examined under a light microscope after staining with hematoxyline and eosin (Drury and Wallington, 1980).

Assessment of liver chemistry: Serum Alanine Aminotransferase (ALT): The activity of this enzyme was estimated by the method of Reitman and Frankel (1957). The assay tubes were labeled blank and sample. To the blank, 0.1ml of distilled water and 5ml of solution Rl were added, while to the sample, 0.lml of the serum and 0.5ml of solution RÌ were added respectively. The mixtures were allowed to incubate for exactly 30 minutes at 37^0C. After incubation, 0.5ml of solution R2 was added to the blank and sample tubes and the mixtures were allowed to stand for 20 minutes at 25°C. Thereafter, 5ml of sodium hydroxide was added to the tubes. Absorbance was read at 546 nm after 5 minutes.

Serum Aspartate Aminotransferase (AST): By the method of Reitman and Frankel (1957). The assay tubes were labeled blank and sample. To the blank, 0.lml of distilled water and 5ml of reagent RI were added while to the sample, 0.1ml of the serum and 0.5ml of reagent RI were added respectively. The mixtures were allowed to incubate for exactly 30 minutes at 37^0C. After incubation, 0.5ml of reagent R2 was added to the blank and sample tubes and the mixtures were allowed to stand for 20 minutes at 25^0C. Thereafter, 5ml of sodium hydroxide was added to the tubes. The absorbance was read at 546 nm after 5 minutes.

Serum Alkaline phosphatase (ALP): By the method of Englehardt (1970). The assay bottle contained 0.02ml of serum and 1ml of the reagent. The mixture was read initially at 405 nm and afterward at 1, 2 and 3 minutes respectively. ALP activity is calculated as 2760 x Δ 405nm/min (U/1).

Serum Total Bilirubin: By the method of Jendrassik and Grof (1938). The assay tubes were labeled blank and sample. To the blank, 200ul of reagent 1, 1000 ul of reagent 2 and 1000 ul of reagent 3 were added and to the sample, 200ul of reagent 1, 50ul of reagent 2, 1000ul of reagent 3 and 200ul of serum were added. The mixtures were allowed to incubate for 10 minutes at 20 - 25°C. Thereafter, 1000ul of reagent 4 was added to the blank and sample tubes respectively. The mixtures were further incubated for 10 minutes at 25°C and the absorbance was measured at 578 nm.

Albumin: By the method of Doumas *et al.* (1971). The assay tubes were labelled blank, standard and sample. To the blank, 3ul of distilled water and 1000 ul of reagent l were added. To the standard, 3ul of the standard reagent and 1000ul of reagent 1 were added and to the sample, 3ul of serum and 1000ul of reagent 1 were added. The mixtures were allowed to incubate for 10 minutes at 37°C. The absorbance of the sample (A sample) and of the standard (A standard) against the reagent blank were measured within 60 minutes at 578 nm.

Statistical Analysis: Results obtained were expressed as mean ±SEM (standard error of mean). Values were considered statistically significant if P value was less than 0.05 ($p < 0.05$).

RESULTS AND DISCUSSION

The results show that administration of arsenic trioxide caused significant ($P<0.05$) serum increase in the activities of alanine aminotransferases and alkaline phosphatase (ALT and ALP) of the Wistar rats. There was significant ($p>0.05$) decrease in the activities of serum aspartate aminotransferases (AST) after the administration of arsenic trioxide. The research also shows that there are significant ($p<0.05$) reduction in the serum level of total protein, albumin and globulin and statistically significant ($p>0.05$) increase in serum

level of total bilirubin after the administration of arsenic trioxide to the Wistar rats. Treatment with ethanolic extract of *Irvingia gabonensis* at doses of 250 and 500 mg/kg significantly decreased serum activities of alkaline phosphatase and total bilirubin and increase in aspartate aminotransferase (table 1)

These are suggestive of damage to the liver (portal hepatitis) by arsenic trioxide, which are further collaborated by the histopathological findings such as periportal infiltrates of inflammatory cells, vascular ulceration, ductal epitheliosis and focal necrosis. Administration of ethanolic extract of *Irvingia gabonensis* ameliorated both the histopathological and biochemical manifestation in the liver. In conclusion, the results from the histopathological analyses and serum liver enzymes biochemical parameters suggested the damage of arsenic trioxide and the potential of the ethanolic leaves extract of *Irvingia gabonensis* in ameliorating the liver damage as revealed in the plates below.

Table 1: Effect of Ethanolic Extract of *Irvingia gabonensis* on liver chemistry serum markers.

Parameters	Group A	Group B	Group C	Group D	Group E	Group F	P-values
ALP	305.8 ± 44.34	738.5 ± 47.28	584.1 ± 55.43	615.0 ± 100.9	443.1 ± 50.15	494.3 ± 40.45	0.0008
AST	95.20 ± 14.03	70.00 ± 2.85	110.7 ± 10.12	102.6 ± 13.35	140.6 ± 36.97	108.0 ± 4.18	0.3106
ALT	74.80 ± 33.01	269.0 ± 35.73	190.1 ± 20.54	92.40 ± 38.93	192.9 ± 15.81	253.7 ± 18.85	< 0.0001
TB	0.2200 ± 0.02	0.300 ± 0.032	0.2571 ± 0.02	0.2400 ± 0.03	0.2429 ± 0.02	0.2333 ± 0.02	0.2713
TP (g/dl)	7.700 ± 0.16	6.100 ± 0.19	6.200 ± 0.12	6.440 ± 0.20	6.486 ± 0.23	6.767 ± 0.02	< 0.0001
Albumin (g/dl)	4.240 ± 0.13	3.500 ± 0.095	3.471 ± 0.06	3.480 ± 0.12	3.486 ± 0.11	3.667 ± 0.02	< 0.0001
Globulin (g/dl)	3.460 ± 0.05	2.600 ± 0.095	2.729 ± 0.08	2.960 ± 0.08	3.000 ± 0.14	3.100 ± 0.03	< 0.0001

ˣP<0.05 indicates significant difference from control

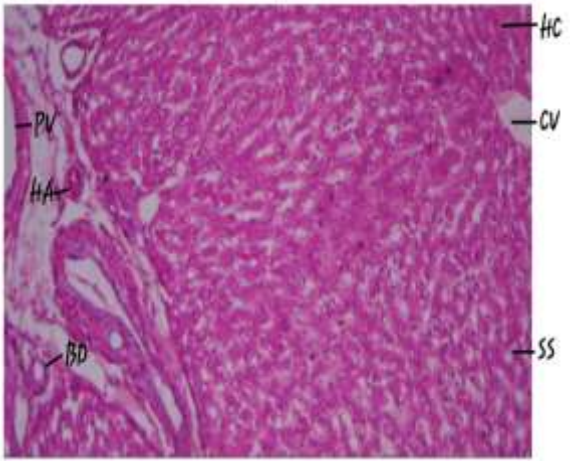

Plate 1. Rat liver. Control. Composed of normal architecture: hepatocytes (HC), sinusoids (SS), central vein (CV), portal vein (PV), hepatic artery (HA), bile duct (BD): H&E 100x.

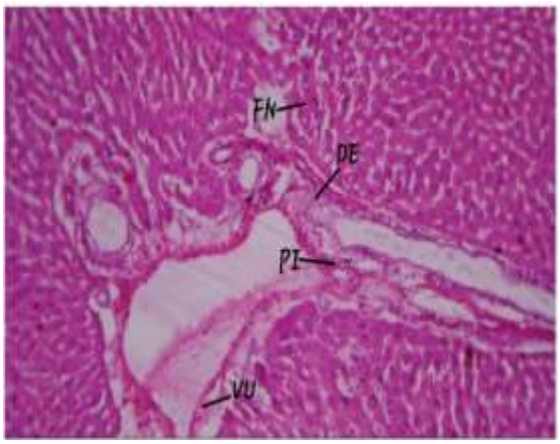

Plate 2. Rat liver exposed to Arsenic trioxide only showing: periportal infiltrates of inflammatory cells (PI), vascular ulceration (VU), ductal epitheliosis (DE), focal necrosis (FN): H&E 100x.

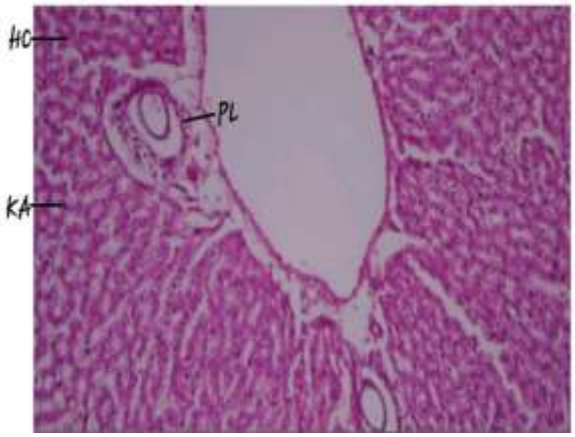

Plate 3. Rat liver exposed to low dose extract plus Arsenic trioxide showing: normal hepatocytes (HC), periportal mobilization of lymphocytes (PL), kupffer cell activation (KA): H&E 100x.

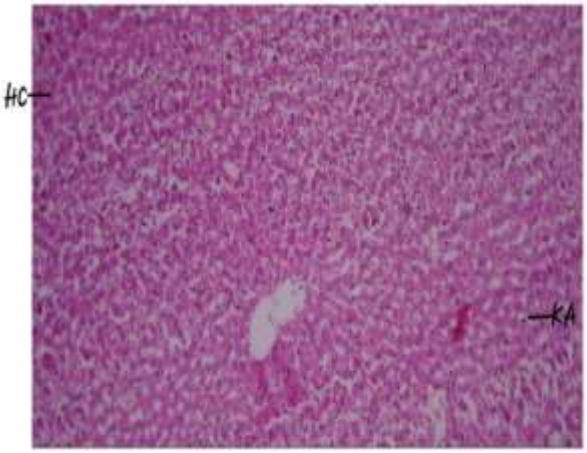

Plate 4. Rat liver exposed to high dose extract plus arsenic trioxide showing: normal hepatocytes (HC), kupffer cell activation (KA): H&E 100x.

Liver markers like alanine transaminase (ALT), alkaline phosphatase (ALP), total bilirubin (TB), conjugated bilirubin (CB) and albumin revealed significant increase in the rats treated with arsenic trioxide when compared with the control. Similar findings were also presented by Wing-Yan (2008). This increase is suggestive of damage to the liver tissue, probably due to hemolysis in the red blood cells and the binding effect of arsenic trioxide to haemoglobin which could lead to inhibition of haem synthesis pathway.

Being a potential sulfhydryl-reactive compound, arsenic trioxide combines with thiol groups of proteins/enzymes in the liver and undergoes biotransformation, thereby interfering with the integrity of hepatic plasma membrane leading to leakage of AST and ALT in serum (Milian *et al.*, 2017).

The purpose of measuring ALT and ALP is to indicate any damage to hepatic cells and problem with bones/gallbladder/kidney. ALT is known to be primarily localized inside liver cells; however, ALP is present in a wide variety of tissues including liver, bones, intestines, kidneys, and other organs. The levels of ALP and ALT are increased to some extent in most cases of liver injury or inflammation. This study has shown that serum levels of ALP and ALT have been significantly increased in arsenic trioxide induced Wistar rats compared to the control. Elevated levels of these enzymes indicated liver dysfunction in induced rats as demonstrated in reports by Milian *et al.*, (2017). The vascular ulceration and ductal epitheliosis revealed by this study further collaborated with the study of Labadie *et al.* (1990) which suggested that arsenic induced hepatic injury is caused by both vascular and hepatocellular damage. In addition, the damage to the hepatic tissue revealed in this study may again be due to overwhelming free radicals or availability of little antioxidant since free radical injury can affect many organs in the body irrespective of their origin and location. The cellular membrane can be damaged and thus cell contents can be released into the blood. The extent of damage depends on the severity of the membrane damaged. It could also inhibit the activity of various enzymes that are important for cellular function.

In conclusion, concurrent treatment with graded doses of *Irvingia gabonensis* achieved a remarkable measure of amelioration, with the lose dose appearing to have a better amelioration effect, thus contributing to knowledge that ethanolic leaves extract of *Irvingia gabonensis* exhibits hepatoprotective activity probably through its bioactive components with antioxidant properties, ability to reduce plasma lipid and could be a potential hepatoprotective substance as revealed by this study.

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